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Original Research Article

Pathogenic activity of *Fusarium equiseti* from plantation of citrus plants (*Citrus nobilis*) in the village Tegal Wangi, Jember Umbulsari, East Java, Indonesia

Dalia Sukmawati* and Mieke Miarsyah

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Campus A, Jl. Rawamangun Muka East Java. Hasyim Ashari Building, 9th floor, Indonesia.

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**Corresponding author email:*
Dalia-Sukmawati@unj.ac.id

Abstract

Some fungi associate with fruit and dead or dying plant tissues as pathogen on a wide range of agricultural plants. This work comprised the isolation, identification and pathogenic assay from citrus fruit plantations (*Citrus nobilis*), Tegal Wangi, Jember, Jawa Timur, Indonesia with 34 mold isolates obtained. Color of 7-day-old colonies cultures on PDA was dominated by white while the reverse was whitish to pale yellow. Based on the pathogenicity test, four representative mold isolates were identified as pathogenic fungi using the sequence of internal transcribed regions Spacer (ITS) in the region of ribosomal DNA selected. Molds were identified as UNJCC (D5) D5K3A (*Fusarium equiseti* with 98% homology bootstrap value 100%), UNJCC (D6) D6. K3.B (*F. equiseti* with 99% homology bootstrap value of 100%), and UNJCC (D7) D7.K2.B (*F. equiseti* with 99% homology bootstrap value 66%) and UNJCC (D8) D3.K2.B (*F. equiseti* with 99% homology bootstrap value of 55%). *F. equiseti* is a main source of trichothecenes, zearalenone and other mycotoxins which can cause serious disease in humans and animals. Present information regarding the *Fusarium equiseti* damage to citrus leaves can be used help identify the occurrence of pathogenic fungi in citrus fruit plantations.

Keywords: *Citrus nobilis*, *Fusarium equiseti*, Pathogenicity, ITS rDNA region

Introduction

Orange is one of the main crop in the village of Tegal Wangi, Indonesia which can help improve the well-being of its citizens in terms of economic development. Constraints faced by the farmers is a decrease in quality of citrus caused by mold destroyer (Sangwanich, 2013). Many citrus plants around agricultural land had suffered damage of tree trunks as brownish, mongering, leaves and fruits having wrinkles with black spots around on their surface. Mold cause serious losses annually on citrus fruit (Ghuffar et al. 2017). *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium*, *Alternaria* and *Mucor* species are major disease source in citrus (Akhtar et al., 2013). Semangun (2007) reported *Fusarium* and *Aspergillus* in citrus fruits. Moscoso-Ramirez et al. (2013) reported mold *Penicillium digitatum* which resulted in whole green fruit and damaged stem with rotten fruit harmful to human health (Sangwanich et al., 2013; Sperandio et al., 2015. Plant diseases are of interest due to wide range of pathogens present in the rhizosphere especially fungi such as *Colletotrichum* sp. (Than et al. 2008; Cannon et al. 2012), *Fusarium*

spp. (Tewoldemedhin et al. 2011), *Fusarium culmorum, F. Oxysporum, F. Sporo-trichioides, Alternaria alternata, A. tenuissima, A. arborescens, A. Infectoria* (Lee et al. 2005), *Alternaria* spp. (Serdani et al. 2002).

Apple trees are susceptible to wide variety of pathogens such as *Fusarium equiseti* (Alonso et al., 2015). Johnston (2008) isolated and identified a wide range of *Penicillium* molds from *Litchi chinensis* Sonn in South Africa using rDNA ITS regions. Wani (2011) reported *Colletotrichum coccodes, C. dermatum,* and *C. gleosporoides* cusing anthracnose in tomato fruits. Damage inflicted blackish-colored black wounds with concave pink colored mycelium growing (Rodrigues and Menezes 2005; Merr et al., 2013). Thiyam and Sharma (2013) showed fungal diseases from local fruits containing *Aspergillus, Acremonium, Alternaria, Aspergillus, Chalaropsis, Cladosporium, Curvularia, Fusariumm, Mucor, Penicillium, Rhizopus* and *Trichoderma* in all fruits during storage. In rainy season, the loss of production in infected fruits by molds can reach 100% (Arauz, 2000). Mold attacking citrus has become one of the limiting production factor in world citrus production (Timmer et al., 2000; Poppe et al., 2003). Fungi a universal pathogen that causes diseases on many fruits such as mango, papaya, and apple and especially in wither caused on citrus due to this pathogen. In this study isolation, identification and testing of highly pathogenic mold of citrus plantation of Siam in The Tegal Wangi Village, the control efficiency of yeasts isolate against pathogenic fungi from citrus leaves was investigated.

Materials and Methods

Sampling location

Location of the sampling site was Desa Krangkongan, Village Tegal Sari, Jember Umbul Wangi, East Java, Indonesia (Fig. 1). Samples were collected from seven citrus trees located in the four corners and center of the total area from orchards (Bagyaraj and Rangaswami, 2007).

Isolation of fungi

For isolation of fungi from citrus plants, the agar method was applied. Fungal pathogens responsible for disease were isolated from leaf surface collected from sampled trees. From the samples obtained, serial dilutions (1:10) were prepared in test tubes with 9 ml sterile water, adding 1 g of leaf samples (previously

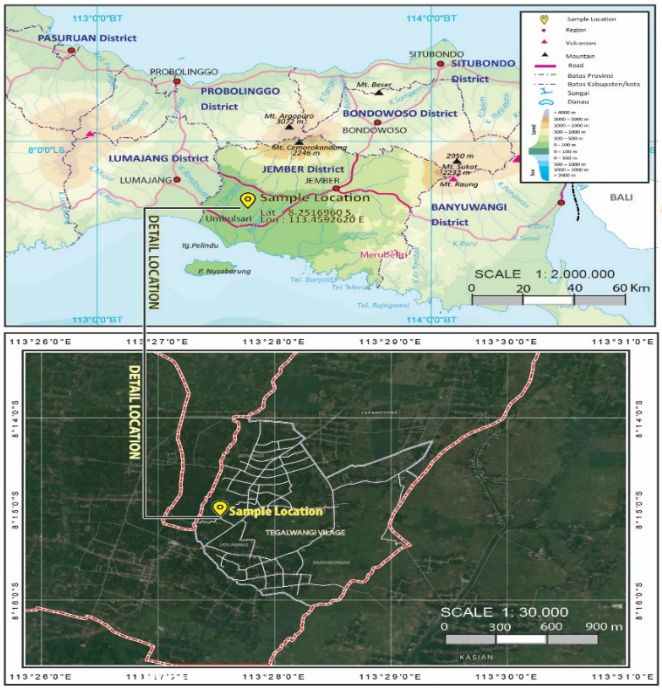

Fig. – 1: Sampling location

sieved) until dilutions 10^{-5}, 10^{-6} and 10^{-7}, of which 50 µL and shaked at 200 rpm for 3 hours. Samples collected were seeded by duplicate through diffusion technique on Petri dishes of 90 mm diameter containing one of the following artificial Potato Dextrose Aga (PDA) and Czapecks agar medium. After incubation (25°C for 7 days) the number for each fungi was calculated. All fungi were cultured using a single spore technique on PDA and Czapecks medium. After incubation, fungi were identified by their macroscopic characters such as colony color, pigment production and mycelium characteristics, and through their microscopic characteristics like the presence of spores and arrangement of sporulation structures examined with a compound microscope (Carl Zeiss) at 1000X. All fungi were subcultured on PDA medium and stored at 4°C. Other isolation methods were also performed based on Farrag (2011) with modifications. Isolation was performed with mold using agar on infected leaves. We used a modified dental needle to the hilt. Each piece of medium was incubated on PDA medium at room temperature (± 30°C).

Purification of Pathogens

Pathogen fungal cultures obtained were purified by the single spore isolation method (Choi et al. 1999). Pure cultures were maintained on PDA slants for further study and preserved with L-drying method in the

University Negeri Jakarta Culture Collection (UNJCC).

Identification of macroscopic and microscopic fungi

Fungal cultures identification was based on macroscopic characteristics like colony morphology, color, texture, shape and appearance and microscopic characteristics like conidia shape, hyphe color, concentric zone, and pigmentation (Navi et al. 1999).

Pathogenicity test

Pathogenicity test used the agar smear method based on the principle of Koch's postulates. Parameter in testing was based on the measurement of disease incidence and severity using the formula described earlier (Embaby et al., 2013). Stages of the pathogenic test included sterilization of leaf surface and inoculation the pathogenic fungi on leaves. Leaf surface sterilization was performed by washing citrus leaves using sterilized water then soaked in a solution of sodium hypochlorite (NaOCl) 0.5% for one minute and further soaked in 70% alcohol for one minute before rinsing with sterilized water. Inoculation of the pathogenic fungi was done with an agar smear method based on Chutia et al. (2009)**.** Test fungi were inoculated with 5mm mycelium plugs from 7-days-old cultures and observation was for 10 days at a temperature of 25-27^{o}C. Growth of fungal species was recorded after one week of incubation and the percentage inhibition was computed after comparison with the control. Lime leaves were placed with 99% moisture and placed in the plastic tubs containing fruits before incubation. Observation was recorded after 10 days when kept at 25-27^{o}C (Agrios, 2005).

Identification of fungi using rDNA sequence

Identification of pathogenic fungi was done using the rDNA on ITS region described by White et al. (1990). Reaction mixture contained specific primers for ITS (Internal Transcribed Spacer region) rDNA region with primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and primer ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'). PCR reaction using PuReTaq™ Ready-To-Go™ PCR Beads (GE Healthcare) total reaction 25 µL, each reaction contained: 15 µL nuclease free water (NFW) dilution in PuReTaq^{TM} Ready-To-Go (RTG) PCR beads (GE Healthcare), 10 pmol primer ITS4 and ITS5 (100 ng DNA template). PCR condition: denaturation at 95°C for 2 min (1 cycle); post denaturation at 94°C for 15 sec, annealing at 56°C for 30 sec, extension at 68°C for 1 min (40 cycles); and 70°C for 10 min in final extension (1 cycle) (Sukmawati et al. 2015). All PCR results were visualized using UV transilluminator after electrophoresis through a 1% agarose gel and ethidium bromide staining. PCR products were sent to 1stBASE (Malaysia) for sequencing.

Phylogenetic analysis

Nucleotide sequence datasets were automatically aligned using the MUSCULE program. Multiple alignments were carried out in MEGA6 (Molecular Evolutionary Genetics Analysis Version 6.0) (Tamura et al. 2007) and sequences retrieved from NCBI (http://www.ncbi.nlm.nih.gov). Phylogenetic analysis was conducted using the maximum likelihood (ML) method in MEGA6. ML analysis was tested by bootstrap (BS) analysis using 1000 replications. BS values of 50% or higher were shown and NR 130661 *Candida orthopsilosis* ATCC 96139 were used as outgroups.

Result and Discussion

Isolation of fungi

Physically observation of citrus fruits showed light brown color with bark peeling and brittle. Plant leaves were developing mold pathogen with blackish leaf spots blackish, speckled blotch, freckle spot, hard spot (shot-hole spot), yellowish, blackish sooty mold developed on the leaves or fruit fouled. Leaves turned yellowish but larger veins remained slightly green which easily fell (Fig. 2). Citrus spp. often got affected by fungal pathogens causing heavy fruit losses (Stammler et al. 2013). General symptoms of citrus plant infected by pathogenic fungi included leaf spots and chlorosis (Teixeira et al. 2005). Five plant pathogenic fungi such as *Alternaria alternata, Rhizoctonia solani, Curvularia lunata, Fusarium oxysporum* and *Helminthosporium oryzae* can infect citrus with black spots on infected leaves (Chutia et al., 2009). Plant pathogen included *Zygomycetes, Ascomycetes* and *Basidiomycetes* (Sukmawati, 2016; Teixeira et al. 2005). Fungi need nutrient from plant for their metabolism.

A total of 34 fungal isolates from citrus leaves was obtained showed diverse morphology of molds on stems and leaves. Mold isolates from the leaves were white (38.2%); light ochre-flesh (26.5%); flesh (8.8%); ochre (5.9%) and others (20.6%) (Table 1).

Mold isolated from leaves had sporulation and pigments (Sukmawati, 2016). Citrus is affected by several mold colors like green affecting fruit quality responsible for major postharvest problems like market losses. Their colors helped in preliminary identification like green and blue mold infections were caused by *Penicillium* spp. (Akhtar et al. 2013) and brown by *Colletotrichum gloeosporioides* Penz (Chung et al. 2002)

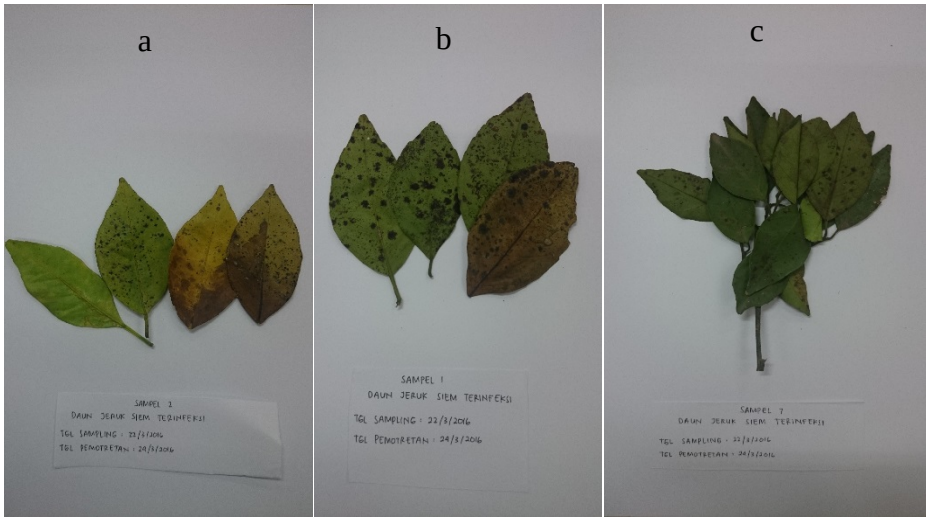

Fig. – 2: Citrus leaves infected with pathogen fungi a: leaves from first plant; b: leaves from second plant and c: leaves from third plant

Table – 1: Morphology of molds isolates on Potato Dextrose Agar (PDA), 3 days' incubation at 27--28^{0}C.

No.	Morphology colony of molds					
	Code of isolate (UNJCC)	**Colours**	**Margin**	**Sporulation**	**Reverse of colony**	**Diameter (L/W) cm**
1	D2. K1. B	White	White	Olive Green	Light Flesh	19.29 / 19.24
2	D3.K2. B	White	White	-	Cinnamon	25.97 / 23.72
3	D4. K1. A	White	White	Olive Green	Cinnamon	26.78 / 30. 46
4	D4. K1. B	White	White	Olive Green	Burnt ochre	22.33 / 26.84
5	D5. K1. A	White	White	Cinnamon	Ochre	31.06 / 32.70
6	D6. K3	white	White	-	light flesh	26.06 / 24.84
7	D7.K2. A	White	White	Cedar Green	white	28.15 / 28.32
8	D7.K2. B	White	White	-	Light Flesh	26.25 / 27.54
9	D7. K3	White	White	-	Light Ochre	77.69/60.30
10	D8. SP. K1. B	White	White	Grey green	White	37.44 / 39.29
11	D8.SP.K2. A	White	White	-	White	27.69 / 25.60
12	D8.SP.K2. B	White	White	Cedar green	White	46.66 / 52.96
13	D8. K1	White	White	-	Cream	82.68/45.00
14	D1. K3	Light Ochre	Light Ochre	-	Gold Ochre	42.25 / 62.77
15	D2. K1. A	Light Ochre	Warm Grey I	Cedar Green	Cinnamon	18.74 / 20.32
16	D3. K1	Light ochre	White	-	Light Ochre	72.84 / 82.36
17	D2. K2	Light Flesh	Cinnamon	-	Light Ochre	62.85/55.00
18	D5. K1. B	Light Flesh	White	-	Light Ochre	31.21 / 29. 78

19	D5. K2	Light flesh	White	Olive Green	Cinnamon	37.35 / 46. 86
20	D7. K1	Light Flesh	White	-	Light Ochre	81.62/46.00
21	D8.K2. A	Light Flesh	White	Light ochre	Ochre	45.47 / 79.17
22	D8.K2. B	Light Flesh	White	Ivory	Light Ochre	46.43 / 81.84
23	D6. K1. A	Medium flesh	Dark flesh	Juniper Green	Light Flesh	46.99 / 76.46
24	D6. K1. B	Medium flesh	Dark flesh	-	Ochre	39.59 / 60.07
25	D6. K2	Medium flesh	Light flesh	-	Light ochre	42.45 / 72.42
26	D3. K3	Ochre	Light Ochre	-	Gold ochre	44.07 / 75.75
27	D8. K3	Ochre	White	-	Gold Ochre	82.5/45.67
28	D1. K1. A	Cinnamon	Cold grey I	-	Light Flesh	21.45 / 21.26
29	D1. K1. B	Caput Mortum	Silver	-	Cold Grey II	18.09 / 19.42
30	D1.K2. A	Burnt Ochre	Brown Ochre	Warm Grey II	Raw Umber	51.81 / 58.22
31	D2. K3	Dark Flesh	White	-	Light Flesh	27.06 / 25.92
32	D3.K2. A	Gold ochre	White	-	Cinnamon	26.54 / 24.64
33	D5. K3	Cedar green	White	-	Olive green	27.96 / 18.74
34	D8.SP. K1.A	Olive green	White	Soft black	Juniper green	55.33 / 65.60

Mold isolated from citrus leaves showed 14 isolates (42%) with color variations among others; green, brown, and black (Table 1). Leaf is one of the source of nutrients and living place of mold isolates. Molds of citrus plants had been characterized by the colony with sporulation such as black, brown, green and yellow greenish (Chutia et al. 2009; Mohammed et al. 2013; Nasiru et al. 2015). Spores are asexual structures in the mold which is useful in deployments to the host (Akhtar et al. 2013; Sperandio et al. 2015). Mold pathogen had various colors like white, light, dark fleash, medium flesh ochre ivory, gold, cadmium and Hyalin (Akhtar et al. 2013; Chutia et al. 2009; Mohammed et al. 2013; Nasiru et al. 2015).

Pathogenicity test

Selection of 8 representative isolates was based on ability of sporulation. The representative mold consisted UNJCC (D1) D8.SP.K1.B; UNJCC (D2) D5. K1. A; UNJCC (D3) D4.K1.B; UNJCC (D4) D8.SP.K1.A; UNJCC (D5) D5.K3.A; UNJCC (D6) D6.K3.B ; UNJCC (D7) D7.K2.B; and UNJCC (D8) D3.K2.B. According to postulant Koch the pathogenicity test showed that four molds isolate were pathogenic (Table 2; Fig. 3). The value of the disease incidence and disease severity was indicated the highest by the isolate with code (D8) D3.K2. B (85%; 100%) (Fig. 3).

Test results proved that the four isolates with original mold causing damage in citrus leaves by highly pathogenic in accordance of Koch's postulates. According to Carla and Renata (2012), Koch's postulates can be used as a criterion of highly pathogenic isolates of a mold. The principle of Koch's postulates consists of: 1) Isolates can be isolated from the diseased host; 2) Isolates can be grown in the laboratory; 3) Isolate the results of isolation will give the same symptoms of the disease on the host, if re-inoculation; 4) Isolates will have the same morphology.

Table – 2: The pathogenicity test results 8 representatives of pathogen mold incubation 10 day at 30 ^{0}C

Isolate Code (UNJCC)	Characteristic of observation (day)				
	d0	d1	d3	d5	d10
(D1)D8. SP. K1. B	Green leaves	Green leaves	-	-	-
(D2)D5. K1. A	Green leaves	Green leaves	-	-	-
(D3) D4. K1. B	Green leaves	Green leaves	-	-	-
(D4) D8. SP. K1. A	Green leaves	There are several causes of brown spots	The color of the leaves becomes brown to black	The color of the leaves becomes brown to black	The color of the leaves becomes brown to black
(D5) D5. K3. A	Green leaves	There are several causes of brown spots	The tips of leaves first, but gradually the dark coloring	The tips of leaves first, but gradually the dark coloring	The tips of leaves first, but gradually the dark coloring
(D6)D6. K3. B	Green leaves	There are several causes of brown spots	The tips of leaves first, but gradually the dark coloring with growing mycelium = 7.28 mm	The tips of leaves gradually the dark coloring with growing mycelium = 8.45 mm	The leaves first, but gradually the dark coloring
(D7) D7.K2. B	Green leaves	There are several causes of brown spots	The leaves become brown to dark with growing mycelium length = 11.59 mm; width = 2.54 mm	The leaves become brown to dark with growing mycelium with white in the margin, with length = 13,61 mm; width = 3,83 mm	The leaves become brown to dark with growing mycelium with white in the margin, with length = 15.61 mm; width = 4,83 mm
(D8) D3.K2. B	Green leaves	There are several causes of brown spots	The leaves become brown to dark with growing mycelium length = 14.29 mm; width = 4.32 mm	The leaves become brown to dark with growing mycelium length = 15.72 mm; width = 4.39 mm	The leaves become brown to dark with growing mycelium length = 17.72 mm; width = 6.39 mm

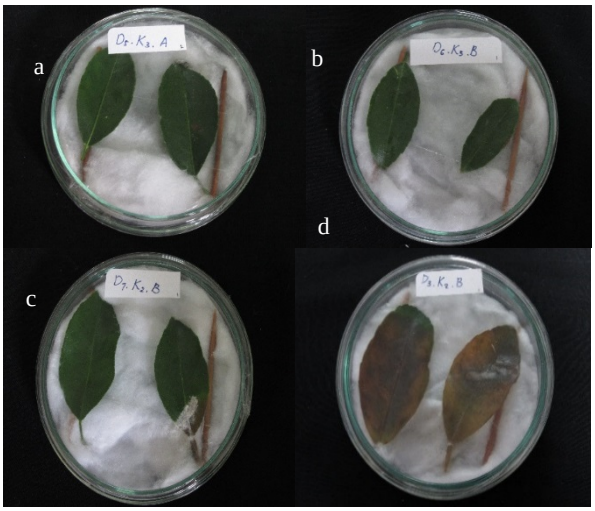

Fig. – 3: Pathogenicity test a: UNJCC (D5) D5.K3.A; b: UNJCC (D6) D6.K3.B ; c: UNJCC (D7) D7.K2.B and d: UNJCC (D8) D3.K2.B incubation 10 day.

Macroscopic observation and identification of molecular ITS region

Identification of molecular sequence analysis done using ITS rDNA region. PCR results on isolates obtained mold band with long bases 600 bp (Fig. 4).

Based on ITS regions of rDNA sequence data, four pathogenic isolates mold consisted following species as UNJCC (D5) D5K3A (*F. equiseti*) with similarity values 98%; UNJCC (D6) D6. K3. B (*F. equiseti*); UNJCC (D7) D7.K2.B (*F. equiseti*); and UNJCC (D8) (D3.K2.B) (*F. equiseti* and *F. oxysporum* sp. *fragariae*) with similar values 99%, which indicated high similarity to their closest species (Fig. 5, Table 4). Based on phylogenetic analysis, four isolates were found for *F. equiseti.*

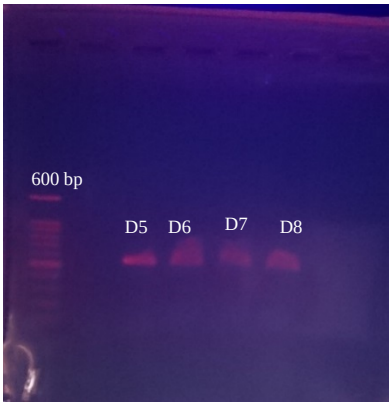

Fig. – 4: Electrophoresis results UNJCC (D5) D5.K3.A; UNJCC (D6) D6.K3.B ; UNJCC (D7) D7.K2.B and UNJCC (D8) D3.K2.B

Table – 3: Identification result of mold isolates from the leaves of citrus from Tegal Wangi Village based on ITS region of rDNA.

Isolate code (UNJCC)	Closely related species	Max scores	Total score	Query score	E-value	Similarity (%)	Accession number
(D5) D5K3A	*F. equiseti*	953	953	97%	0	98%	AY928409.
(D6) D6K3B	*F. equiseti*	1018	1018	99%	0	99%	KX588103.
(D7) D7K2.B	*F. equiseti*	987	987	98%	0	99%	KX588103.
(D8) D3.K2B	*F. equiseti*	1050	1050	99%	0	99%	KR364600.

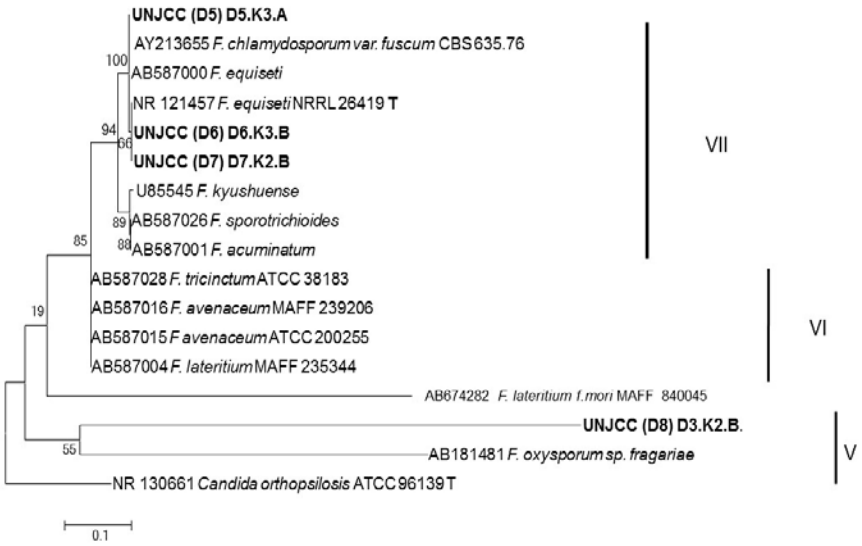

Fig. – 5: Maximum likelihood tree showing taxonomic position of *Fusarium* strains isolated from orange leaves: UNJCC (D5) D5.K3.A; UNJCC (D6) D6.K3.B; UNJCC (D7) D7.K2.B and UNJCC (D8) D3.K2.B. The tree was rooted to NR 130661 *Candida orthopsilosis* ATCC 96139

The *Fusarium* genome was first described by Link in 1809 (Aoki et al., 2014). Based on the phylogenetic tree, isolates (D5) D5.K3.A; (D6) D6.K3.B; (D7) D7.K2.B and (D8) D3.K2.B were identified as one clade with *F. equiseti* with bootstrap value of 94% (Fig. 5). The low value of the need for this data described the bootstrap analyzed by using a gene other than ITS rDNA. These regions have high success rates to identify a molecular approach (Schoch et al. 2012). But not all isolates of *Fusarium* can be identified are accurate based on a single gene. Isolate fungi with code (D8) D3.K2.B are identified as *F. equiseti*. While according to phylogenetic analysis isolate (D8) D3.K2.B was one clade with AB181481 *F. oxysporum* sp. *fragariae.* Watanabe et al. (2011) reported seven clade in *Fusarium.* Our riset consist of clade VII, clade VI and Clade V. Clade VI consists of *F. lateritium, F. avenaceum,* and *F. tricinctum,* which belong to different "sections", namely, *Lateritium, Roseum,* and *Sporotrichiella* respectively. The paraphyly of *F. avenaceum and F. lateritium* was supported by all the genes. Clade VII contains 4 "sections" with 9 species: Eupionnotes consisting of *F. incarnatum, Gibbosum consisting of F. equiseti* and *F. acuminatum, F. graminearum and F. culmorum,* and *Sporotrichiella* consisting of *F. poae, F. kyusyuense, F. sporotrichioides,* and *F. langsethiae*. Clade V consisting *F. oxysporum sp. fragariae*. (Watanabe et al. 2011). *Fusarium* species is known one of the most difficult species to be identified based on morphological markers among fungal species. One of the main reasons for this difficulty is that genetic and morphological characters vary among strains in a species and the ranges of character diversity are often overlapped among closely-related species. Although all fore gene trees supported the classification of *Fusarium* species into 7 major clades, I to VII.

According to Tunarsih et al. (2015) suggested to use suitable marker for the identification of *Fusarium* members as *Fusarium* genome has possibly unique evolutionary history. Watanabe et al. (2011) used multigene analysis for *Fusarium* genome (18S rDNA gene, ITS1, 5.8S rDNA, 28S rDNA, *β*-tubulin gene, and aminoadipate reductase gene (*lys2*) for inter-species identification of *Fusarium*. Their results showed that sequence has homology with bootstrap value of 65–100%. *F. equiseti* mold can cause damage to various crops, including corn, rice and wheat in field and storage (Hasem et al. 2010). Palmero et al. (2011) reported that all the tested *Fusarium* isolates were pathogenic on tomato and melon. Regarding Bakar et al. (2013), *Fusarium* species are one of the common pathogens of post-harvest disease to cause rot on tomato and other perishable vegetable fruits. A total of 180 *Fusarium* isolates were obtained from 13 locations throughout Selangor. *Fusarium solani* was the most abundantly isolated (34%) followed by *F. semitectum* (31%) and *F. oxysporum* (31%), *F. subglutinans* (3%) while the last was *F. equiseti* (1%).

Fusarium equiseti is a plant pathogenic fungus and produce secondary metabolites form toxins that can be pathogenic on the various plants on agricultural land (Kosiak et al. 2005). Secondary metabolites produced vary in amount and toxicity. This species produces a variety of toxins, such as trichothecenes type A, for example, neosolaniol (NEO), diacetoxy-scirpenol (DAS), the type of T-2 toxin and HT-2, type B trichothecenes, for example, nivalenol (NIV), and non-essential compounds such as trichothecene zearalenon (ZEA), equisetin and fusarochromanone (Barros et al. 2012). In addition, it can also be profitable. This fungi have potential infection in rooting plants (Macia-Vicente et al. 2008) and the special nature of belonging so that could make these fungi as a candidate for biological control of nematodes (Nitao et al. 2001; Horinouchi et al. 2007). Other potential owned by mold, *F. equiseti* i.e. capable of producing the enzyme xyloglucanases (XG) (Rashmi & Siddalingamurty, 2016). These enzymes are known to have potential in processing waste plant, modifications to improve the nature of xyloglucans reologi in the food industry and the feed, fabric treatment to change the brightness and color, to remove the fuzz from the surface of textile materials in the textile industry and the paper industry (Sinitsyna, 2010). The enzyme is easily obtained so easily applied to help lower the cost of production.

Conclusion

Sampling mold pathogen in citrus plant plantation was done in isolation of pathogenic mold by direct and washing methods. Isolation retrieved as many as 34 isolates derived from leaf mold- Mold colonies from the leaves and stems were dominated by white colonists with mold. Testing of highly pathogenic samples was made from leaves of citrus plantation in Jember. Testing was conducted on eight isolates of highly pathogenic representative molds. Four potentials isolate mold caused the same damage when symptoms mold isolates from diseased leaves. These isolates were UNJCC (D5) D5. K3. A; UNJCC (D6) D6. K3. B; UNJCC (D7) D7. K2. B; and UNJCC (D8) D3. K2. B. Based on their phylogenetic analysis, all isolates were identified as *F. equiseti* (UNJCC (D5) D5K3A with 98% homology bootstrap values 64%, isolate UNJCC (D6) D6. K3. B 99% homology with bootstrap values 100%, isolate UNJCC (D7) D7. K2. B 99% homology with bootstrap values 99%, and UNJCC (D8) D8 D3. K2. B 99% homology with bootstrap values of 88%.

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